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Cyclosporin Analogs for the Treatment of Lung Diseases

Technical Field

The present invention relates to novel cyclosporin analogs and methods for the treatment of asthma and other diseases characterized by airflow obstruction in a subject. The present invention further relates to pharmaceutical compositions comprising the compounds of the present invention and processes for their production.

Background of the Invention

Respiratory diseases, such as asthma and other diseases characterized by airflow obstruction, are a global problem. Millions of people worldwide, both children and adults, suffer from these medical conditions. These diseases reduce quality of life by impairing the ability of sufferers to perform everyday tasks, and in some cases, cause death. One of the major respiratory diseases is asthma.

Asthma is a disease of unknown etiology in which the bronchi are inflamed and as a consequence obstructed. This narrowing results from a combination of bronchial smooth muscle contraction, mucosal oedema, inflammatory cell infiltrate and partial or total occlusion of the lumen with mucus, cells and cell debris. Bronchial obstruction is either partially or totally reversible, and this important feature distinguishes asthma from chronic bronchitis.

Asthma is an extremely common disease with a worldwide prevalence of 5% to 8%. In the developed world it is the most common chronic illness and, for reasons that are unclear, the disease is on the increase. It is now accepted that

asthma is a chronic inflammatory disorder of the airways in which many cells play a role, in particular, mast cells, eosinophils and T-lymphocytes. In susceptible individuals this inflammation causes symptoms which are usually associated with widespread but variable airflow obstruction. This type of airflow obstruction is often reversible either spontaneously or with treatment and causes associated increase in airway responsiveness to a variety of stimuli.

The illness has a wide clinical spectrum ranging from mild episodic bronchospasm (easily controlled by the occasional use of a bronchodilator) to a very severe intractable asthma sometimes resistant to treatment with high doses of oral corticosteroids. Steroid resistance occurs in less than 5% of people with asthma. This translates to thousands of people. These patients with severe chronic disease may be dependent on corticosteroids and their disease is often so severe that full reversibility can be difficult or impossible to demonstrate.

Chronic obstructive airways disease, chronic obstruction lung disease and 'smoker's chest' have all been used to describe what is now known as COPD. COPD is characterized by progressive irreversible airway obstruction. It can lead to death from respiratory or cardio-respiratory failure. COPD consists of two subsets: chronic bronchitis and emphysema. In practice, it is very difficult to define the contribution of each of these two conditions to the obstruction of the airway and this has led to the displacement of these labels by the non-specific term COPD. The pathology of COPD is not fully elucidated, but features include hypertrophy of mucus-secreting glands, inflammation (including infiltration with lymphocytes) and goblet cell hyperplasia.

The treatment of COPD consists of bronchodilators, intermittent courses of antibiotics and, in some patients, inhaled and/or oral corticosteroids. The latter is claimed to reduce the decline in lung function in COPD.

Cystic fibrosis is an inherited condition. Excess viscid mucus is produced. This leads to recurrent chest infections and progressive bronchiectasis. Approximately 50% of cystic fibrosis sufferers have bronchial hyperresponsiveness and there is an increased incidence of atopy. There is widespread airway narrowing and wheeze. Most cystic fibrosis sufferers take bronchodilators, some take inhaled corticosteroids. And at least one study had reported benefit with oral corticosteroids.

Current drugs for treating asthma are corticosteroids (such as beclomethasone, triamcinolone), beta adrenergics (such as epinephrine, albuterol, bitolterol), NSAIDS, leukotriene antagonists, Xanthines (methyl xanthines such as theophylline, oxtriphylline) and anticholinergics (such as atropine, ipratropium bromide).

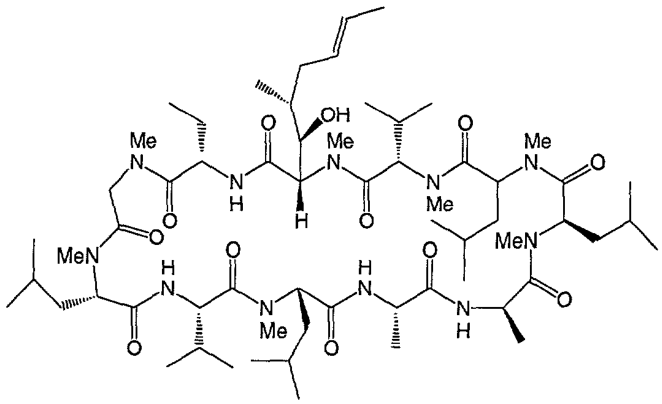
Corticosteroids are the mainstay of treatment of chronic asthma and they revolutionized the treatment of this disease when they were first introduced in the 1950's. Oral corticosteroids have today been largely replaced by inhaled corticosteroids, although severe asthmatics still require medication by mouth. Inhaled cortisteroids are relatively safe and extremely effective in most patients, and improved the quality of life for millions of asthmatic sufferers. For those with severe asthma, however, oral therapy with corticosteroids is required. When taken for more than a few days oral corticosteroids have a number of serious side effects. These include growth retardation in children, severe osteoporosis (especially in old age), decreased responsiveness of the pituitary adrenal axis to stress, fluid retention, diabetes and precipitation of psychosis.

Furthermore, an appreciable number of patients have apparent corticosteroid resistance or unreponsiveness. Patients considered successfully treated with inhaled or oral steroids often have to be content with 60% of their predicted lung function. Further increasing the dose of oral corticosteroids runs the risk of concomitant side effects.

Although corticosteroids are effective for asthma, they are not ideal drugs. Over the years doctors have occasionally used immunosuppressive agents as adjuncts to corticosteroids in patients with extremely severe disease. Examples of immunosuppressive drugs are azathioprine, methotrexate, mycophenolic acid and prodrug, leflunomide, Cyclosporin A, ascomycin, FK-506 and rapamycin.

The cyclosporins comprise a class of structurally distinctive, cyclic, poly-N-methylated undecapeptides, commonly possessing pharmacological activity, in particular immunosuppressive, anti-inflammatory or anti-parasitic activity. The first of the cyclosporins to be isolated was the naturally occurring fungal metabolite

cyclosporin, *Cyclosporin A* represented as follows:



Since the original discovery of cyclosporin, a wide variety of naturally occurring cyclosporins have been isolated and identified, and many further non-natural cyclosporins have been prepared by total- or semi-synthetic means or by the application of modified culture techniques. The class comprising cyclosporins is thus now substantial and includes, for example, the naturally occurring Cyclosporins A through Z, for example, [Thr]^{2}, [Val]^{2}, [Nva]^{2} and [Nva]$^{2-}$, [Nva]^{5}-Cyclosporin (also known as Cyclosporins C, D, G and M respectively), [(D)MeVal]^{11}-Cyclosporin (also known as Cyclosporin H), [cf., Traber et al.;1, Helv. Chim. Acta, 60, 1247-1255 (1977); Traber et al.; 2, Helv. Chim. Acta, 65, 1655-1667 (1982); Kobel et al.; Europ. J. Applied Microbiology and Biotechnology, 14, 273-240 1982); and Von Wartburg et al.; Progress in Allergy, 38, 28-45, 1986)]; as well as various non-natural cyclosporin derivatives and artificial or synthetic cyclosporin derivatives and artificial or synthetic cyclosporins including dihydrocyclosporins [in which the MeBmt-residue is saturated by hydrogenation]; derivatized cyclosporins (e.g., in which the 3'-O-atom of the MeBmt- residue is acylated or a further substituent is introduced at the α-carbon atom of the sarcosyl residue at the 3-position); and cyclosporins in which variant amino acids are incorporated at specific positions within the peptide sequence, for example, [3-O-acetyl-MeBmt]^{1}–Cyclosporin (also known as Dihydro-cyclosporin D), [(D)Ser]^{8}-Cyclosporin, [MeIle]^{11}-Cyclosporin, [MeAla]^{6}.Cyclosporin, [(D) Pro]^{3}-Cyclosporin etc., employing the total synthetic method for the production of cyclosporins developed by R. Wenger—see e.g. Traber et al., 1; Traber et al., 2; and Kobel et al., loc cit. U.S. Pat. Nos. 4,108,985, 4,220,641, 4,288,431, 4,554,351, 4,396,542 and 4,798,823; European Patent Publication Nos. 34,567A, 56,782A, 300,784A and 300,785; International Patent Publication No. WO 86/02080 and UK Patent Publication Nos. 2,206,119 and 2,207,678; Wenger 1, Transpl. Proc., 15 Suppl.

1:2230 (1983); Wenger 2, Angew. Chem. Int. Ed. 24 77 (1985) and Wenger 3, Progress in the Chemistry of Organic Natural Products, 50, 123 (1986).

There is increasing evidence that chronic inflammation in asthma is mediated via a network of cytokines emanating from inflammatory and structural cells in the airways. The prominent eosinophilic inflammation that characterizes asthma appears to be orchestrated by cytokines derived from type 2 T-helper (Th2)-like lymphocytes, suggesting that immunosuppressants might be beneficial in the control of asthma (*see* for example, "Pharmacokinetics, pharmacodynamics, and safety of inhaled cyclosporin A after single and repeated administration in healthy male and female subjects and asthmatic patients," Rohatagi, S. et al., Aventis Pharmaceutical, Collegeville, PA, USA. J. Clin. Pharmacol. (2000), 40(11), 1211-1226). Cyclosporin A (hereinafter "CsA") is active against CD4+ lymphocytes and might, therefore, be useful for asthma. A trial of low-dose oral CsA in patients with steroid-resistant asthma indicated that it can improve control of symptoms in patients with severe asthma on oral steroids.

The mechanism of CsA action in asthma is of interest. CsA binds to the ubiquitous protein cyclophilin, in the cytosol, and the complex in turn binds to calcineurin, which is a calcium and calmodulin dependent serine threonine phosphatase. Calcineurin is necessary for the cytoplasmic portion of the transcription factor NF-AT, a nuclear factor of activated T-cells, to translocate to the nucleus and bind to its nuclear portion to become an active transcription factor. NF-AT forms a complex with AP-1 and regulates the transcription of the IL-2 gene, together with other genes, for example, IL-5. CsA prevents the cytoplasmic portion of NF-AT from translocating, resulting in reduced transcription of IL-2. CsA has a specific inhibitory effect in CD4+ cells through this transcription mechanism, but may also have inhibitory effects on other cells, including mast cells and eosinophils, through mechanisms that have not yet been defined.

Recently, three controlled trials of CsA in asthma have been reported. [Alexander AG, Barnes NC, Kay AB. Trial of cyclosporin in corticosteroid-dependent chronic severe asthma. *Lancet* **1992**; 339: 324-328; Niwanowska E, Dworski R, Domala B, Pinis G. Cyclosporin for steroid-dependent asthma. *Allergy,* **1991**; 46: 312-315; Lock SH, Kay AB, Barnes NC. Double-blinded, placebo-controlled study of cyclosporin A as a corticosteroid-sparing agent in corticosteroid-dependent asthma. *Am J Respir Crit Care Med* **1996**; 153: 509-14; Nizankowska E, Soja J, Pinis G, Bochenek G, Sladek K, Domagala B, et al. Treatment of

steroid-dependent bronchial asthma with cyclosporin. *Eur Respir J* **1995**; 8: 1091-1099.]

CsA 5 mg/kg/day allowed a significant reduction in the use of corticosteroids by 60%. Side effects with systemic CsA were increase in diastolic blood pressure and decrease in renal function. Other side effects include hepatic dysfunction, hypertrichosis, tremor, gingival hyperplasis and paraesthesia. The systemic toxicity of CsA limits its use for the treatment of asthma, COPD and other related lung diseases. Therefore, it is desirable to synthesize analogs of CsA which retain CsA's potential utility as a primary or adjunct therapy for respiratory diseases, while reducing or eliminating CsA's systemic toxicity.

<u>Summary of the Invention</u>

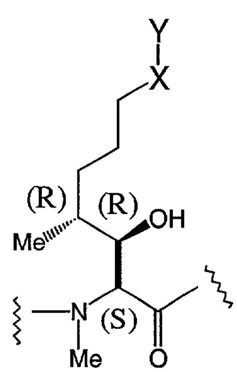
The present invention relates to novel cyclosporin analogs and methods of treatment for the treatment of asthma and other diseases characterized by airflow obstruction in a subject. The present invention further relates to pharmaceutical compositions comprising the compounds of the present invention and processes for their production.

More particularly, the present invention relates to a cyclosporin analog of the following formula (I) or a pro-drug or pharmaceutically acceptable salt thereof:

A---B---Sar-MeLeu-Val-MeLeu-Ala---U---MeLeu-MeLeu-MeVal
1 2 3 8 11

(I)

In formula I, the formula for residue A is:



where X is absent, -C1-C6 alkyl-, or –C3-C6 cycloalkyl-; Y is selected from the groups: -C(O)-O-R1; -C(O)-S-R1; -C(O)-OCH_2-OC(O)R2; -C(S)-O-R1; and -C(S)-S-R1; where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio or halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio and where R2 is C1-C6 alkyl optionally substituted with halogen, C1-C6 alkoxy, C1-C6 alkylthio heterocyclics or aryl; B is -αAbu-, -Val-, -Thr- or -Nva-; and U is –(D)Ala-, -(D)Ser- or –[O-(2-hydroxyethyl)(D)Ser]-, or -[O-acyl(D)Ser]- or –[O-(2-acyloxyethyl)(D)Ser]-.

In a second embodiment, the present invention relates to the use of the cyclosporin analogs of the present invention or a pro-drug or a pharmaceutically acceptable salt thereof in pharmaceutical compositions for the treatment of asthma and other diseases characterized by airflow obstruction in a subject.

In a third embodiment, the present invention relates to processes for the production of novel cyclosporin analogs of the present invention. In a preferred embodiment, the present invention relates to the processes for the production of cyclosporin analogs of formula I, with the structure of residue A as illustrated above.

The present invention also contemplates method(s) of treatment of asthma and other diseases characterized by airflow obstruction in a subject by administering to the subject therapeutically effective amounts of the cyclosporin analogs of the present invention with or without the concurrent use of other drugs or pharmaceutically acceptable carriers or excipients.

<u>Detailed Description of the Invention</u>

The present invention relates to novel cyclosporin analogs and methods of treatment for the treatment of asthma and other diseases characterized by airflow obstruction in a subject. The present invention further relates to pharmaceutical compositions comprising the compounds of the present invention and processes for their production. The patents and publications identified in this specification indicate the knowledge in this field and are hereby incorporated by reference in their entirety. In the case of inconsistencies, the present disclosure will prevail.

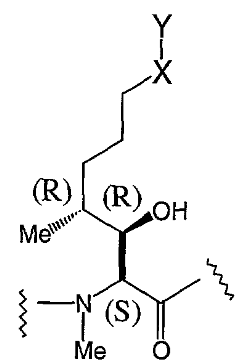
More particularly, the present invention relates to a cyclosporin analog of the following formula (I) or a pro-drug or pharmaceutically acceptable salt thereof:

A---B---Sar-MeLeu-Val-MeLeu-Ala---U---MeLeu-MeLeu-MeVal

1 2 3 8 11

I

In formula I, the formula for residue A is:



where X is absent, -C1-C6 alkyl-, or –C3-C6 cycloalkyl-; Y is selected from the groups: -C(O)-O-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy or halogen substituted C1-C6 alkylthio; -C(O)-S-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy or halogen substituted C1-C6 alkylthio; -C(O)-OCH$_2$-OC(O)R2 where R2 is C1-C6 alkyl optionally substituted with halogen, C1-C6 alkoxy, C1-C6 alkylthio heterocyclics or aryl; -C(S)-O-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy or halogen substituted C1-C6 alkylthio; and -C(S)-S-R1 where R1 is hydrogen, C1-C6 alkyl optionally substituted with halogen, heterocyclics, aryl, C1-C6 alkoxy or C1-C6 alkylthio, halogen substituted C1-C6 alkoxy, halogen substituted C1-C6 alkylthio; B is -αAbu-, -Val-, -Thr- or -Nva-; and U is –(D)Ala-, -(D)Ser- or –[O-(2-hydroxyethyl)(D)Ser]-, or -[O-acyl(D)Ser]- or –[O-(2-acyloxyethyl)(D)Ser]-.

In formula I, abbreviation of amino acid residues, for example, -Ala-, -MeLeu-, -αAbu-, etc., are in accordance with conventional practice and are to be understood as having the L-configuration unless otherwise indicated (for example,

-(D)Ala- represents a residue having the D-configuration). Abbreviation of residues preceded by "Me-" represents a α-N-methylated amino acid residue, for example, "Me-Leu" is a α-N-methylated-Leucine residue. Individual residues of a molecule of the cyclosporin analog of the present invention are numbered, as in the art, clockwise and starting with the residue -MeBmt-, corresponding to residue 1. The same numerical sequence is employed throughout the present specification and claims.

In a most preferred embodiment, a cyclosporin analog of the present invention is represented by formula I or a pro-drug or pharmaceutically acceptable salt thereof, where residue B is -αAbu- and residue U is -(D)Ala-. In another preferred embodiment, the cyclosporin analog of the present invention is represented by formula I or a pro-drug or pharmaceutically acceptable salt thereof, where X is absent in residue A, residue B is -αAbu- and residue U is - (D)Ala-.

Representative compounds of the invention include, but are not limited to, the following compounds as illustrated below:

Compound of formula I, where in residue A, X is absent and Y = -$COOCH_3$; residue B = -αAbu-, and residue U = -(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -COOH; residue B = -αAbu-, and residue U = -(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -COOEt; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2CH_2CH_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2Ph$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2F$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$COOCHF_2$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$COOCF_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2CF_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2Cl$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2OCH_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2OCH_2CH_2O$ CH_3; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -C(=O)SCH_2Ph; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is -$CH_2CH_2CH_2$- and Y = -$COOCH_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

Compound of formula I, where in residue A, X is absent and Y = -COOFmoc; residue B = –αAbu-, and residue U = –(D)Ala-.

Cyclosporin analogs of the invention are accordingly useful for the treatment of diseases or conditions responsive to or requiring topical anti-inflammatory, immunosuppressive or related therapy, for example, topical administration for the treatment of such diseases or conditions of the eye, nasal passages, buccal cavity, skin, colon or, especially, airways or lung. In particular, cyclosporin analogs of the invention permit topical anti-inflammatory, immunosuppressive or related therapy with the concomitant avoidance or reduction of undesirable systemic side effects, for example general systemic immunosuppression.

Cyclosporin analogs of the invention useful for the treatment of diseases and conditions of the airways or lung, in particular, inflammatory or obstructive airway diseases. They are especially useful for the treatment of diseases or conditions of the airways or lungs associated with or characterized by inflammatory cell infiltration or other inflammatory events accompanied by inflammatory cell accumulation, for e.g., eosinophil and/or neutrophil. Most preferably, they are useful for the treatment of asthma.

Cyclosporin analogs of the invention are useful in the treatment of asthma of whatever type of genesis including both intrinsic and, especially, extrinsic asthma. They are useful for the treatment of atopic and non-atopic asthma, including allergic asthma, bronchitic asthma, exercise induced asthma, occupational asthma, asthma induced following bacterial infection and other non-allergic asthmas. Treatment of asthma is also to be understood as embracing treatment of "wheezy-infant syndrome," that is treatment of subjects, for example, of less than 4 to 5 years of age, exhibiting wheezing symptoms, in particular at night, and diagnosed or diagnosable as "wheezy infants," an established patient category of major medical concern and now more correctly indentified as incipient or early-phase

asthmatics. Cyclosporin analogs of the invention are in particular useful for the treatment of asthma in subjects whose asthmatic status is either steroid dependent or steroid resistant.

Cyclosporin analogs of the invention are also useful for the treatment of bronchitis or for the treatment of chronic or acute airways obstruction associated therewith. Cyclosporin analogs of the invention may be used for the treatment of bronchitis of whatever type or genesis, including, for example, acute bronchitis, arachidic bronchitis, catarrhal bronchitis, chronic bronchitis, croupous bronchitis, phthinoid bronchitis and so forth.

Cyclosporin analogs of the invention are in addition useful for the treatment of pneumoconiosis (an inflammatory, commonly occupational, disease of the lungs, frequently accompanied by airways obstruction, whether chronic or acute, and occasioned by repeated inhalation of dusts) of whatever type or genesis, including, for example, aluminosis, anthracosis, asbestosis, berylliosis, chalicosis, ptilosis, siderosis, silicosis, tabacosis and, in particular, byssinosis.

Cyclosporin analogs of the invention may also be used for the treatment of eosinophil-related disorders of the airways (e.g. involving morbid eosinophilic infiltration of pulmonary tissues) including hypereosinophilia as it effects the airways and/or lungs as well as, for example, eosinophil-related disorders of the airways consequential or concomitant to Loffler's syndrome, eosinophilic pneumonia, parasitic (in particular metazoan) infestation (including tropical eosinophilia), bronchopulmonary aspergillosis, polyarteritis nodosa (including Churg-Strauss syndrome), eosinophilic granuloma and eosinophil-related disorders affecting the airways occasioned by drug-reaction.

The word "treatment" as used herein in relation to the treatment of diseases of the airways and lungs, in particular asthma, is to be understood as embracing both symptomatic and prophylactic modes, that is for immediate treatment, for e.g., of acute inflammation (symptomatic treatment) as well as for advance treatment to prevent, ameliorate or restrict long term symptomatology (prophylactic treatment). The term "treatment" as used in the present specification and claims in relation to such diseases is to be interpreted accordingly as including both symptomatic and prophylactic treatment, for e.g., in the case of asthma, symptomatic treatment to ameliorate acute inflammatory events and prophylactic treatment to restrict on-

going inflammatory status and to ameliorate future bronchial exacerbation associated therewith.

Cyclosporin analogs of the invention may also be used to treat any disease or condition of the airways or lungs requiring immunosuppressive therapy, for e.g., the treatment of autoimmune diseases, or as they affect, the lungs (for example, for the treatment of sarcoidosis, alveolitis or chronic hypersensitivity pneumonitis) or for the maintainance of allogenic lung transplant, for e.g., following lung or heart lung transplantation.

As previously indicated, for the above purposes, cyclosporin analogs of the invention will be administered topically within the airways, for e.g., by the pulmonary route or by inhalation. As also previously noted, while having potent efficacy when administered topically, cyclosporin analogs of the invention exhibit reduced systemic toxicity. Cyclosporin analogs of the invention thus provide a means for the treatment of diseases and conditions of the airways or lung, for example, as hereinabove set forth, with the avoidance of unwanted systemic side effect, e.g. consequent to inadvertent swallowing of drug substance during inhalation therapy. It is estimated that during the course of manoeuvres required to effect administration by inhalation, up to 90% or more of total drug substance administered will normally be swallowed rather than inhaled.

By the provision of cyclosporin analogs which are topically active, e.g. effective when inhaled, but systemically inactive the present invention makes cyclosporin therapy available to subjects for whom such therapy might otherwise be excluded, e.g. due to the risk of systemic, in particular immunosuppressive, side effect.

Further uses include the treatment and prophylaxis of inflammatory and hyperproliferative skin diseases and cutaneous manifestations of immunologically-mediated illnesses, such as psoriasis, atopical dermatitis, contact dermatitis and further eczematous dermatitises, seborrhoeis dermatitis, Lichen planus, Pemphigus, bullous pemphigoid, Epidermolysis bullosa, urticaria, angioedemas, vasculitides, erythemas, cutaneous eosinophilias, Lupus erythematosus, acne and Alopecia areata; various eye diseases (autoimmune and otherwise) such as keratoconjunctivitis, vernal conjunctivitis, keratitis, herpetic keratitis, conical cornea, dystrophia epithelialis corneae, corneal leukoma, ocular pemphigus, Mooren's ulcer, Scleritis, Graves' opthalmopathy, Vogt-Koyanagi-Harada

syndrome, sarcoidosis, multiple myeloma, etc.; obstructive airway diseases, which includes conditions such as COPD asthma (for example, bronchial asthma, allergic asthma, intrinsic asthma, extrinsic asthma and dust asthma), particularly chronic or inveterate asthma (for example, late asthma and airway hyper-responsiveness), bronchitis, allergic rhinitis and the like; inflammation of mucosa and blood vessels such as gastric ulcers, vascular damage caused by ischemic diseases and thrombosis. Moreover, hyperproliferative vascular diseases such as intimal smooth muscle cell hyperplasia, restenosis and vascular occlusion, particularly following biologically- or mechanically-mediated vascular injury can be treated or prevented by the compounds of the invention.

The compounds of the present invention may also find utility in the chemosensitization of drug resistant target cells. Cyclosporin A and FK-506 are known to be effective modulators of P-glycoprotein, a substance which binds to and inhibits the action of anticancer drugs; by inhibiting P-glycoprotein, they are capable of increasing the sensitivity of multidrug resistant (MDR) cells to chemotherapeutic agents. It is believed that the compounds of the invention may likewise be effective at overcoming resistance expressed to clinically useful antitumour drugs such as 5-fluorouracil, cisplatin, methotrexate, vincristine, vinblastine and adriamycin, colchicine and vincristine.

Accordingly, the pharmaceutical compositions of the present invention comprise a therapeutically effective amount of a cyclosporin analog of the invention in combination with a pharmaceutically acceptable carrier or excipient. In particular, compositions pertaining to the present invention are useful for treating a subject for a reversible obstructive airway disease.

The present invention also contemplates method(s) of treatment of asthma and other diseases characterized by airflow obstruction in a subject by administering to the subject therapeutically effective amounts of the cyclosporin analogs of the present invention with or without the concurrent use of other drugs or pharmaceutically acceptable carriers or excipients, as described throughout the present specification. Such treatment of the disease may be done by administering a therapeutically effective amount of a compound of the invention for such time and in such amounts as is necessary to produce the desired result.

As used in the present invention, "therapeutically effective amount" of one of the compounds means a sufficient amount of the compound to treat a particular

disease, at a reasonable benefit/ risk ratio. The compounds of the present invention may be employed in pure form or, where such forms exist, in pharmaceutically acceptable salt, ester or prodrug forms. Alternatively, the compound may be administered as pharmaceutical compositions containing the compound of interest in combination with one or more drugs or pharmaceutically acceptable excipients. It will be understood, however, that the total daily usage of the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment.

The specific therapeutically-effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts. For example, it is well within the skill of the art to start doses of the compound at levels lower than required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved.

Dosages of the cyclosporin analogs of the present invention employed in practicing the method of the present invention will of course vary depending on the site of treatment, the particular condition to be treated, the severity of the condition, the subject to be treated (for e.g., in terms of body weight, age and so forth) as well as the effect desired. In general, for treating diseases or conditions of the airways or lungs, for e.g., inflammatory or obstructive airway disease such as asthma, cyclosporins of the invention can be suitably administered topically to the airways or lungs, for e.g., but not limited to, inhalation, at dosages from about 20 to about 400 mg/day, preferably from about 50 to about 300 mg/day, most preferably from about 200 to about 300 mg/day. Dosages will appropriately be administered from a metered delivery system in a series of from 1 to 5 puffs at each administration, with administration performed once to four times daily. Dosages at each administration will thus conveniently be from about 5 to 100 mg/day, more preferably from about 12.5 to about 100 mg/day, e.g. administered with a metered delivery device capable of delivering, for e.g., 1 to 25 mg cyclosporin per actuation. For purposes of oral administration, more preferable doses may be in the range from about 0.005 to about 3 mg/kg/day. If desired, the effective daily dose may be

divided into multiple doses for purposes of administration; consequently, single dose compositions may contain such amounts or submultiples thereof to make up the daily dose.

Definitions

The terms "C_1-C_3-alkyl" or "C_1-C_6-alkyl" as used herein refer to saturated, straight- or branched-chain hydrocarbon radicals containing between one and three or one and six carbon atoms, respectively. Examples of C_1-C_3 alkyl radicals include methyl, ethyl, propyl and isopropyl, and examples of C_1-C_6-alkyl radicals include, but are not limited to, methyl, ethyl, propyl, isopropyl, *n*-butyl, *tert*-butyl, neopentyl and n-hexyl.

The term "C_1-C_6-alkoxy" as used herein refers to an C_1-C_6-alkyl group, as previously defined, attached to the parent molecular moiety through an oxygen atom. Examples of C_1-C_6-alkoxy include, but are not limited to, methoxy, ethoxy, propoxy, isopropoxy, *n*-butoxy, *tert*-butoxy, neopentoxy and n-hexoxy.

The term "C_1-C_6-alkylthio" as used herein refers to an C_1-C_6-alkyl group, as previously defined, attached to the parent molecular moiety through a sulfur atom. Examples of C_1-C_6-alkylthio include, but are not limited to, thiomethoxy, thioethoxy, thiopropoxy, thio-isopropoxy, *n*-thiobutoxy, *tert*-thiobutoxy, neothiopentoxy and n-thio-hexoxy.

The term “aryl” as used herein refers to a carbocyclic ring system having one or more aromatic rings including, but not limited to, phenyl, naphthyl, tetrahydronaphthyl, indanyl, indenyl and the like. Aryl groups (including multi-cyclic aryl groups) can be unsubstituted or substituted with one, two or three substituents independently selected from lower alkyl, substituted loweralkyl, haloalkyl, alkoxy, thioalkoxy, lower alkylenedioxy, lower alkylidenedioxy, amino, alkylamino, dialkylamino, acyamino, cyano, hydroxy, acyl, halo and/or trifluoromethyl, mercapto, nitro, carboxylaldehyde, carboxy, alkoxycarbonyl, carbamoyl, sulfamoyl, lower alkoxycarbonylamino, lower alkanoyl, ureido, amidino and carboxamide. In addition, substituted aryl groups include tetrafluorophenyl and pentafluorophenyl.

The term "C_3-C_6-cycloalkyl-” as used herein refers to carbocyclic groups of 3 to 6 carbons, respectively; for example, cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

The terms "halo" and "halogen" as used herein refer to an atom selected from fluorine, chlorine, bromine and iodine.

The term "heterocyclics", as used herein, refers to a cyclic aromatic radical having from five to ten ring atoms of which one ring atom is selected from S, O and N; zero, one or two ring atoms are additional heteroatoms independently selected from S, O and N; and the remaining ring atoms are carbon, the radical being joined to the rest of the molecule via any of the ring atoms, such as, for example, pyridinyl, pyrazinyl, pyrimidinyl, pyrrolyl, pyrazolyl, imidazolyl, thiazolyl, oxazolyl, isooxazolyl, thiadiazolyl, oxadiazolyl, thiophenyl, furanyl, quinolinyl, isoquinolinyl, and the like.

The term "subject" as used herein refers to a mammal or animal. Preferably the mammal is a human. A subject refers to, for example, dogs, cats, horses, cows, pigs, guinea pigs and the like.

The term "pro-drug" as used herein refers to pharmacologically acceptable derivatives, for example, but not limited to, esters and amides, such that the resulting biotransformation product of the derivative is the active drug. Pro-drugs are known in the art and are described generally in, e.g., Goodman and Gilman's "Biotransformation of Drugs," in the Pharmacological Basis of Therapeutics, 8th Ed., McGraw Hill, Int. Ed. 1992, page 13-15, which is hereby incorporated by reference in its entirety.

As used herein, the term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge, *et al.* describe pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences, 66: 1-19 (1977), incorporated herein by reference. The salts can be prepared *in situ* during the final isolation and purification of the compounds of the invention, or separately by reacting the free base function with a suitable organic acid. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid,

citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, *p*-toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, loweralkyl sulfonate and aryl sulfonate.

Pharmaceutical Compositions

The pharmaceutical compositions of the present invention comprise a therapeutically effective amount of a compound of the present invention formulated together with one or more pharmaceutically acceptable carriers. As used herein, the term "pharmaceutically acceptable carrier" means a non-toxic, inert solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. Some examples of materials which can serve as pharmaceutically acceptable carriers are sugars such as lactose, glucose and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil; safflower oil; sesame oil; olive oil; corn oil and soybean oil; glycols; such a propylene glycol; esters such as ethyl oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminun hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol, and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the

composition, according to the judgement of the formulator. The pharmaceutical compositions of this invention can be administered to humans and other animals orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, or drops), bucally, or as an oral or nasal spray.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polethylene glycols and the like.

The active compounds can also be in micro-encapsulated form with one or more excipients as noted above. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms the active compound may be admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, e.g., tableting lubricants and other tableting aids such a magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

Dosage forms for topical or transdermal administration of a compound of this invention include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants or patches. The active component is admixed under sterile conditions with a pharmaceutically acceptable carrier and any needed preservatives or buffers as may be required.

Pharmaceutically acceptable diluents or carriers may be diluents or carriers acceptable for topical application at the intended site of therapy, e.g. diluents or carriers acceptable for topical administration pulmonary, dermally, nasally, ocularly or rectaly.

The ointments, pastes, creams and gels may contain, in addition to an active compound of this invention, excipients such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to the compounds of this invention, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants such as chlorofluorohydrocarbons.

Transdermal patches have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms can be made by dissolving or dispensing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate can be controlled by either providing a rate controlling membrane or by dispersing the compound in a polymer matrix or gel.

Forms in topically administrable form, e.g. enabling or facilitating topical administration, include, e.g. dry powder preparations of the active ingredient (i.e. cyclosporin analog of the invention) in substantially pure form, for example as employed in the art for delivery from a dry powder inhalation device. Means or devices enabling or facilitating topical administration include, in particular, inhalation devices as well as containers and the like from which the active ingredient may be delivered in a form capable of topical application. Preferred embodiments as defined under C will be such as permit topical administration within the airways or lungs, e.g. by inhalation.

It is clear that safety may be maximized by delivering the drugs by the inhaled route either in nebuliser form or as dry powder. Clearly the great advantage of the inhaled route, over the systemic route, in the treatment of asthma and other diseases of airflow obstruction and/or of chronic sinusititis, is that patients are exposed to very small quantities of the drug and the compound is delivered directly to the site of action.

Preparation of forms suitable for administration by inhalation may be carried out by methods known in the art. It should be noted that several antibiotics have recently developed for topical inhaled usage, particularly in cystic fibrosis, where they have been shown to be effective against pseudomonas infections. Various

inhalants are described. For example, in DE 1491707, GB 1,392,945, GB 1,457,351, GB 1,457,352, NL 147939, DE 1491715, GB 1,598,053, EP 5585, EP 41783, EP 45419, EP 360463 and FR 2628638. DE 1491715, in particular, is said to be suitable for inhalation therapy intended for bronchial or lung diseases.

For this purpose cyclosporin analogs of the invention may be employed in any suitable finely dispersed or finely dispersible form, capable of administration into the airways or lungs, for example in finely divided dry particulate form or in dispersion or solution in any appropriate (i.e. pulmonarily administerable) solid or liquid carrier medium. For administration in dry particulate form, cyclosporin analogs of the invention may, for example, be employed as such, i.e. in micronised form without any additive materials, in dilution with other appropriate finely divided inert solid carrier or diluent (e.g. glucose, lactose, mannitol, sorbitol, ribose, mannose or xylose), in coated particulate form or in any other appropriate form as know in the art for the pulmonary administration of finely divided solids.

Pulmonary administration may be effected using any appropriate system as known in the art for delivering drug substance in dry or liquid form by inhalation, e.g. an atomizer, nebulizer, dry-powder inhaler or like device. Preferably a metered delivery device, i.e. capable of delivering a pre-determined amount of cyclosporin analog at each actuation, will be employed. Such devices are known in the art.

For nasal administration, cyclosporin analogs of the invention will suitably be administered in liquid form from a nasal applicator. Suitable topical forms for the treatment of diseases or conditions of the skin will include, for example, creams, gles, ointments, pastes, cataplasms, plasters, transdermal patches and the like. Formulations for dermal application will appropriately contain a skin penetration enhancer, e.g. as know in the art, for example azone. Forms suitable for ophthalmic use will include lotions, tinctures, gels, ointment and ophthalmic inserts, again as known in the art. For rectal administration, i.e. for topical therapy of the colon, cyclosporin analogs of the invention may be administered in suppository or enema form, in particular in solution, e.g. in vegetable oil or like oily system for use as a retention enema.

According to the present invention, cyclosporin analogs may be used for the manufacture of a topical preparation for the treatment, with or without the concurrent use of other drugs. For the above purposes, cyclosporin analogs of the

invention may be employed in any dosage form appropriate for topical administration to the desired site. For example, for the treatment of diseases of the airways or lungs, cyclosporin analogs of the invention may be administered via the pulmonary route, by inhalation from an appropriate dispenser device.

Dosage for the topical preparation will in general be one tenth to one hundredth, of the dose required for oral preparation.

Abbreviations

Sar:	Sarcosine
MeLeu:	N-Methyl-Leucine
Val:	Valine
Ala:	Alanine
MeVal:	N-Methyl Valine
Et:	Ethyl
Ph:	Phenyl
Fmoc:	9-Fluorenylmethoxycarbonyl-
MeBmt:	N-Methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine
α-Abu:	α-Aminobutyric acid

Synthetic Methods

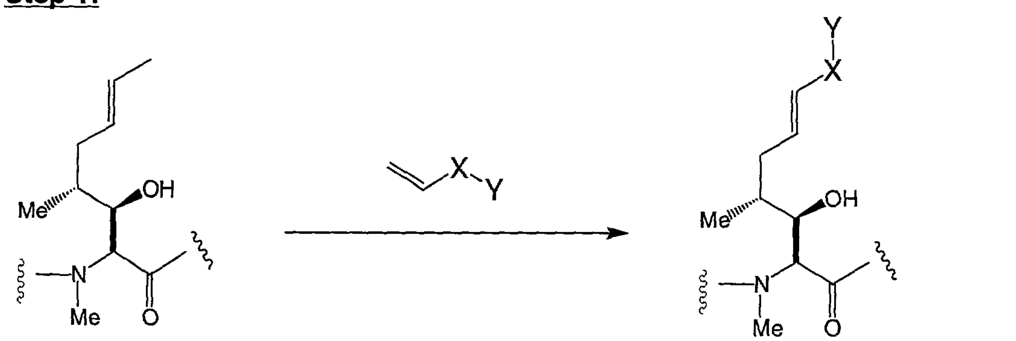
The compounds and processes of the present invention will be better understood, but are not limited to, the following synthetic scheme which illustrates the methods by which the compounds of the present invention (illustrated by formula I) may be prepared. The groups X and Y, and the amino acid residues B and U in formula I are as defined earlier in the specification. The starting material for Scheme I, illustrated by formula I where A'= -MeBmt-, may be, for example, but not limited to, a fermentation product or a synthetic product made by solution phase chemistry. Preferably, the starting material is commercially available. The starting material as a fermentation product may be made from highly productive strains, for example, but not limited to, *Sesquicillopsis rosariensis* G. ARNOLD F605; *Tolypocladium inflatum* wb6-5; Fusant, *Tolypocladium inflatum* KD461 etc. (in U.S. Patent Nos. 5,256,547; 5,856,141 etc.). Alternately, the starting material may be made by solution phase chemistry either by sequentially assembling amino acids or by linking suitable small peptide fragments, where the units are linked by, for example, but not limited to, amide, ester or hydroxylamine linkages (described

in, *Müller, Methoden der organischen*, Chemie Vol. XV/2, pp 1 to 364, Thieme Verlag, Stuttgart, 1974; Stewart, Young, Solid Phase Peptide Synthesis, pp 31 to 34, 71 to 82, Pierce Chemical Company, Rockford, 1984; Bodanszky, Klausner, Ondetti, Peptide Synthesis, pp 85 to 128, John Wiley & Sons, New York, 1976 and other standard books on solution phase peptide chemistry). For amide linkages particular preference is given to the azide method, the symmetric and mixed anhydride method, *in situ* generated or preformed active esters and methods using coupling reagents (e.g., dicyclohexylcarbodiimide, N,N-dimethyl-4-aminopyridine, N-hydroxy-benzotriazole, PyBrop® etc.). Classical solution phase chemistry using standard Z- and Boc- methodology may be used.

Residue A, which is -MeBmt- in the starting material is further modified, as illustrated in the following reaction scheme.

Scheme:

Step 1:



A' = -MeBmt-

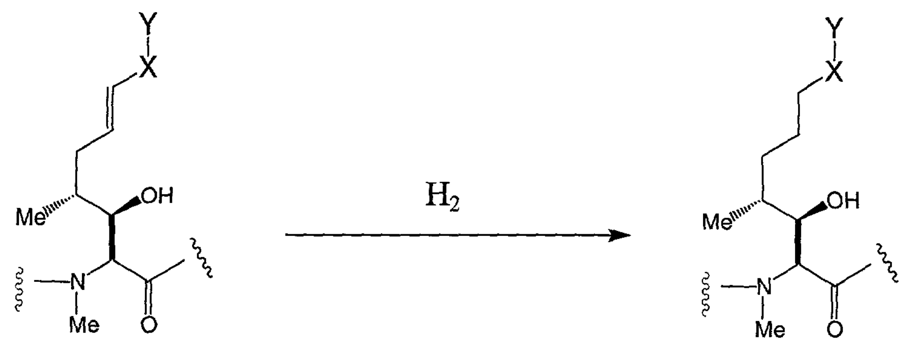
A", wherein X, Y are as defined

(i)

The process for the preparation of the compounds of formula I comprises reacting a compound of formula I, where A' = -MeBmt- (for example, Cyclosporin A) with an olefin having a terminal double bond with catalysts such as Grubb's ruthenium alkylidene, Grubbs dihydroimidazole ruthenium, Shrock-Hoveyda molybdenum catalysts or benzylidene catalysts [*see* (a) US Patent 6,111,121; (b) Reviews: Synlett, **1999**, *2*, 267; (c) Reviews: Ivin, K J; Mol, J.C. *Olefin Metathesis and Metathesis Polymerization*, 2nd ed., Academic Press, New York, **1997**; (d) *J. Org. Chem.*, **1999**, *64*, 4798-4816; (e) *Angew. Chem.*, Int. Ed. English, **1997**, *36*, 2036-2056; (f) *Tetrahedron* **1998**, *54*, 4413-4450.] or Nolan's ruthenium catalyst [see (a) International Patent Application No. WO 00/15339; (b) *Org. Lett.*, **2000**, *2*, 1517-1519; (c) *J. Org. Chem.*, **2000**, *65*, 2204-2207] or Molybdenum catalysts [see

(a) *J. Am. Chem. Soc.*, **1990**, *112*, 3875 (b), *J. Am. Chem. Soc.*, **1996**, *118*, 10926-10927] in the presence of a lithium salt such as lithium bromide, lithium chloride, lithium trifluoroacetate, lithium triflate of a lewis acid such as titanium isopropoxide in an organic solvent. The organic solvent used may be solvents such as, for example, dichloromethane, chloroform, toluene, benzene, tetrahydrofuran, dimethylformamide and the like or mixtures thereof. The reaction may be carried out from room temperature to about 100 °C for 1-7 days to provide a compound of formula I, where residue A' is converted to residue A" having formula (i).

Step 2:



A", wherein X, Y are as defined

(i)

A, wherein X, Y are as defined

The compounds of formula I in an organic solvent, where residue A" has formula (i), are then subjected to standard hydrogenation conditions using a catalyst such as, but are not limited to, a catalytic amount of palladium on carbon in a hydrogen atmosphere to provide the saturated compounds of formula I, where in particular, residue A" having formula (i) is converted to residue A, as described throughout the specification.

The organic solvents used can be solvents such as methanol, ethanol, ethyl acetate or mixtures thereof. Other catalysts useful to assist hydrogenation may be, for example, but not limited to, platinum metal or its oxide [*see* standard books on catalytic hydrogenation, e.g., Rylander, P.N., *Hydrogenation Methods*, Academic Press: NY, 1985; *Catalytic Hydrogenation in Organic Synthesis*, Academic Press: NY, 1985; Červený, L., *Catalytic Hydrogenation*, Elsevier: NY, 1986 etc.]. The reaction may be carried out at room temperature or elevated temperature, for example, but not limited to, 50 °C or 100 °C.

Examples

The procedures described above for preparing the compounds of the present invention will be better understood in connection with the following examples, which are intentended to be illustrative only and not limiting of the scope of the invention. Various changes and modifications of the disclosed embodiments will be apparent to those skilled in the art. Such changes and modifications, including without limitation, those relating to the chemical structures, substituents, derivatives, intermediates, syntheses, formulations and/or methods fo the invention may be made without departing from the spirit of the invention and the scope of the appended claims.

Example 1: Compound of formula I, where in residue A, X is absent and Y = -$COOCH_3$; residue B = -αAbu-, and residue U = -(D)Ala-.

Cyclosporin methyl ester (0.030 mg, 0.024 mmol) and palladium on carbon (0.0012 mg, 0.0012 mmol) were added to a flask and the flask was evacuated and backfilled with hydrogen gas three times. Anhydrous methanol (3 ml) was added and the reaction was stirred for 18 h at ambient temperature under an atmosphere of hydrogen. After filtration and concentration in vacuo, the product was isolated as a white solid (0.021 mg, 70 % yield). Electrospray mass spectrum (ESMS) M+H: 1248.91

Example 2: Compound of formula I, where in residue A, X is absent and Y = -COOEt; residue B = –αAbu-, and residue U = –(D)Ala-.

The title compound of example 2 was prepared from cyclosporin ethyl ester and palladium on carbon according to the procedures described in Example 1. ESMS M+H: 1262.3

Example 3: Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2CH_2CH_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

The title compound of example 3 was prepared from cyclosporin propyl ester and palladium on carbon according to the procedures described in Example 1.

Example 4: Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2Ph$; residue B = –αAbu-, and residue U = –(D)Ala-.

The title compound of example 4 was prepared from cyclosporin benzyl ester and palladium on carbon according to the procedures described in Example 1.

Example 5: Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2F$; residue B = –αAbu-, and residue U = –(D)Ala-.

The title compound of example 5 was prepared from cyclosporin fluoromethyl ester ester and palladium on carbon according to the procedures described in Example 1

Example 6: Compound of formula I, where in residue A, X is absent and Y = -$COOCHF_2$; residue B = –αAbu-, and residue U = –(D)Ala-.

The title compound of example 6 was prepared from cyclosporin difluoromethyl ester ester and palladium on carbon according to the procedures described in Example 1

Example 7: Compound of formula I, where in residue A, X is absent and Y = -$COOCF_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

The title compound of example 7 was prepared from cyclosporin trifluoromethyl ester ester and palladium on carbon according to the procedures described in Example 1.

Example 8: Compound of formula I, where in residue A, X is absent and Y = -$COOCH_2CF_3$; residue B = –αAbu-, and residue U = –(D)Ala-.

The title compound of example 8 was prepared from cyclosporin trifluoroethyl ester ester and palladium on carbon according to the procedures described in Example 1.

The cyclosporin analogs of the present invention have potent immunosuppressive and anti-inflammatory activity. In particular, they inhibit antigen-induced inflammatory cell infiltration, for example, into the airways. In vivo this activity is apparent following topical administration, e.g., pulmonary route.

The immunosuppressive and anti-inflammatory properties of cyclosporin analogs of the invention may be demonstrated in standard test models *in vitro* and *in vivo* for example as follows.

Example 9: Calcineurin Inhibition Assay

The immunosuppressive activity of cyclosporin is mediated through inhibition of the phosphatase activity of the enzyme calcineurin by a cyclophilin-

cyclosporin complex. Thus, calcineurin inhibition is widely used as an *in vitro* measure of the activity of cyclosporin analogs.

Compounds were tested in an assay based on the Biomol Green Calcineurin Assay Kit supplied by Biomol (Plymouth Meeting, PA), supplemented with Cyclophilin A for enzyme inhibition. The activity of the recombinant human calcineurin was determined by release of phosphate from a phosphopeptide representing a fragment of camp-dependent protein kinase. Phosphate release was determined using the colorimetric detection reagent Biomol Green (Biomol AK-111).

Compounds in DMSO (2.4μl) were added to a 96-well microplate and mixed with 50μl assay buffer (50mM Tris-HCl, pH 7.5; 100mM sodium chloride; 6mM magnesium chloride; 0.5mM dithiothreitol, 0.025% NP-40, 500μM calcium chloride, 0.27μM Calmodulin) containing 10μM Cyclophilin and 3nM Calcineurin. After warming to 37 °C for 60 mins, the enzymatic reaction was initiated by addition of phosphopeptide (7.5μl) to give a final concentration of 94μM. Phosphate release after 60 min at 37 °C was determined by addition of Biomol Green (100 μl) and measurement of the absorbance at 620nm after 15 mins at room temperature.

IC_{50} values were calculated from determinations of enzyme activity at inhibitor concentrations ranging from 0.1 to 0.0015 μM.

Example 10. NFAT reporter gene assay

NFAT activation follows precisely the activation of calcineurin by increased free calcium levels in the cytoplasm. Researchers from diverse fields are interested in the NFAT family of transcription factors, which are potential targets for newer and safer immunosuppressive drugs. In addition, the activation of NFAT proteins involves various cellular signal transduction pathways, including calcium mobilization and MAP kinase pathways linked to T-cell receptors and Ras1. To assist researchers probing the activity of NFAT proteins, Stratagene has developed a PathDetect cis-reporter plasmid, the pNFAT-Luc reporter plasmid (Stratagene, Inc. catalog # 219094), containing the NFAT binding site from the human IL-2 gene.2,7-9. The NFAT cis-reporting system includes the transfection-ready pNFAT-Luc reporter plasmid and the pCIS-CK negative control plasmid.

Construction of the pNFAT-Luc Plasmid:

The backbone of the 5749-base-pair pNFAT-Luc plasmid is the pFR-Luc reporter plasmid of the aforementioned PathDetect trans-reporting system. To this backbone, the GAL4 binding element was replaced with four direct repeats of the NFAT binding sequence (–286 to –257) from the IL-2 gene promoter, the most studied and widely used NFAT binding sequence. For all reporter plasmids of the PathDetect cis-reporting systems, activation of the luciferase gene indicated interaction of uncharacterized gene products, extracellular stimuli, growth factors, or drug candidates with specific enhancer elements. Then a plasmid expressing the gene of interest was cotransfected into mammalian cells along with a cis-reporter plasmid to indicate transcription activation.

Testing the pNFAT-Luc Plasmid in Jurkat Cells:

Pharmacology studies have established that NFAT proteins can be activated by the protein kinase C activator phorbol ester (PMA) in combination with the calcium ionophore ionomycin, reagents that raise free intracellular calcium. When Jurkat cells, a mature human T-cell line, or CHO cells were transfected with the pNFAT-Luc plasmid and treated with 60 ng/ml of PMA and 1μg/ml of inomycin, luciferase activity increased by 13- and 16-fold, respectively. Therefore, the enhancer element in the pNFAT-Luc plasmid is responsive to calcium mobilization. Cells transfected with pNFAT-Luc and then treated with either PMA or ionomycin alone did not show a significant increase in luciferase activity.

Cyclosporin inhibits the activity of calcineurin, a protein phosphatase regulated by intracellular calcium mobilization. All the isoforms of NFAT protein contain a calcineurin-binding domain and are activated by calcineurin. The inhibition of luciferase expression from pNFAT-Luc in the present model, in both Jurkat and CHO cells induced by PMA and ionomycin, was monitored for cyclosporin (as a positive control) and the cyclosporin analogs of the present invention.

In another set of experiments, rat basophilic leukemia cells stably transfected with chemokine receptors were transfected with pNFAT-Luc and then treated with their respective ligands (data not shown). When both luciferase expression and calcium levels were monitored in these cells, luciferase expression correlated very well with calcium mobilization. Therefore, luciferase expression from pNFAT-Luc indeed reflects the activation of endogenous NFAT proteins by calcium immobilization.

Example 11. Immunosuppressive Activity and Applications

Murine Mixed Lymphocyte Reaction

Ca. $0.5x10^6$ lymphocytes from the spleen of female (8-10 weeks) Balb/c mice are incubated for 5 days in 0.2 ml cell growth medium with ca. 0.5×10^6 lymphocytes from the spleen of female (8-10 weeks) CBA mice. Test substance is added to the medium at various concentrations. Activity is assessed by ability to suppress proliferation-associated DNA synthesis as determined by incorporation of radiolabelled thymidine.

Mishell-Dutton Test

Ca. 10^7 lymphocytes from the spleen of OFI, female mice are co-cultured with ca. $3x10^7$ sheep erythrocytes for 3 days. Test substance is added to the incubation medium in varying concentrations. Lymphocytes are harvested and plated onto agar with fresh sheep erythrocytes as antigen. Sensitized lymphocytes secrete antibody that coats the erythrocytes, which lyse to form a plaque in the presence of complement. Activity is assessed by reduction in the number of plaque forming, i.e., antibody product, cells.

Delayed-type Hypersensitivity Resonse

On Day 0 groups of ten mice (having BALB/cByJ or any other acceptable strain) are dosed with test compound (1 to 10%), vehicle or the positive control, cyclophosphamide (Cyclosporin A), and monitored from Day-2 to 7. The mice are anesthetized and their abdomens shaved. 100 µl of a 3% solution of ovalbumin are applied to the abdomen and dried. Seven days later, the mice are challenged by applying 5 µl of ovalbumin to each side of the right ear. After 24 hours, both the right and left ear thickness are measured using a micrometer caliper.

Popliteal Lymph Node Assay

First, an inducer (phenytoin) is injected into the mice footpad (having BALB/cByJ or any other acceptable strain). Then the mice are challenged (subcutaneously or po) with ester and control agent using graded doses, for example, 2.5, 10, 20 mg/Kg (based on cyclosporine A data). On day 7 the popliteal lymph nodes are excised from the dosed mice and the lymph nodes are weighed. Then single cell suspensions of each lymph node are prepared and enumerated. The weight index for each animal is calculated (for example, a mean weight index <2 would indicate suppression of immune response).

Influence on Allergen-Induced Pulmonary Eosinophilia (*in vitro*)

Male Himalayan spotted guinea pigs (300 g, BRL) are sensitized to ovalbumin (OA) by i.p. injection of 1 ml of a suspension of OA (10 μg/ml) with $Al(OH)_3$ (100 mg) and B-pertussis vaccine (0.25 ml) in saline (0.9% w/v). For oral studies, the procedure is repeated 1x after 2 weeks and the animals are used one week later. For inhalation studies, the procedure is repeated 2x at 3-week intervals and the animals are used one week after the last injection.

Challenge is effected employing a saline solution of OA, nebulized for discharge into an exposure chamber. Test animals are exposed to OA by nose-only inhalation for 60 minutes. For inhalation studies, OA solution is used at a concentration of 0.01%.

Test substance is administered (a) inhalation and/or (b) orally. For oral studies, test substance is administered p.o. in olive oil 1x daily for 3 days or in powder form in methylcellulose once prior to OA challenge. On day 3, test animals receive test substance 1.5 hrs. prior to and 6 hrs. after OA challenge. For inhalation studies, test substance is micronised for delivery to test animals restrained within a flow-past, nose-only inhalation chamber. Administration by inhalation is effected 15 mins. prior to OA challenge.

Efficacy of administered test substance is determined by bronchoalveolar lavage (BAL) and cell counting. For this purpose animals are sacrificed with Na pento-barbitone (100 mg/kg i.p.) and the trachea is exposed and cannulated. 5 successive 10 ml aliqots of Ca^2+ and Mg^2+ free Hank's balanced salt solution (HBSS), containing bovine serum albumin (BSA, 0.3%), EDTA (10mM) and HEPES (10 mM) is then introduced into the lung and immediately aspirated by gentle compression of the lung tissue. Total cell counts in pooled eluates are determined using an automatic cell counter. Lavage fluid is centrifuged at 200g for 10 minutes and the cell pellet resuspended in 1 ml of supplemented HBSS. 10 μl of this cell suspension is added to 190 μl of Turk's solution (1:20) dilution). Differential cell counts are made from smears stained by Diff-Quick. Cells are identified and counted under oil immersion (x1,000). A minimum of 500 cells per smear are counted and the total population of each cell type is calculated.

In untreated animals, OA challenge induces increase of all cell types in BAL fluid 24 hours after challenge. Prior administration of cyclosporin analogs in

accordance with the present invention by inhalation at dosages of the order of from 1.0 to 15.0 mg/kg reduces eosinophil count in BAL in a dose dependent manner as compared with untreated controls. Cell counts for other leucocytes (macrophages, neutrophils etc.) are also reduced.